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PPARα and γ Activation Effects of New Nor-triterpenoidal Saponins from the Aerial Parts of *Anabasis articulata*

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Key words
PPARα, PPARγ, *Anabasis articulata*, antidiabetic, Amaranthaceae, triterpene saponins

received June 1, 2018
revised October 5, 2018
accepted October 14, 2018

Bibliography
DOI https://doi.org/10.1055/a-0762-0885
Published online | Planta Med © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

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ABSTRACT

Anabasis articulata, traditionally used to treat diabetes, is rich in saponin content. This study was performed to investigate the agonistic effect of its saponins on peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ in human hepatoma (HepG2) cells to explore the possibility of the involvement of these nuclear receptors in the mechanism of the antidiabetic effect of the plant. Chemical investigation of the *n*-butanol fraction resulted in the isolation of three new and one known 30-noroleanane triterpenoid saponins. The structures of the new compounds were elucidated as 3β-hydroxy,23-aldehyde-30-norolean-12,20(29)-dien-28-oic acid-28-*O*-β-D-glucopyranosyl ester (**1**), 3β-*O*-D-galactopyranosyl-23-aldehyde-30-norolean-12,20(29)-dien-28-oic acid-28-*O*-β-D-glucopyranosyl ester (**2**), and 3β-*O*-D-xylopyranosyl-30-norolean-12,20(29)-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**), while the known 30-nortriterpenoidal saponin was identified as boussingoside E (**4**). Although, the isolated saponins (**1**–**4**) did not show > 1.5-fold activation of peroxisome proliferator-activated receptor-γ, but two of them (**1** and **3**) activated peroxisome proliferator-activated receptor-α to the higher extents of 2.25- and 1.86-fold, respectively. These results suggest that the reported antidiabetic action of the isolated saponins may not solely involve the activation of peroxisome proliferator-activated receptor-γ. However, the agonistic activity of the *n*-butanol fraction of *A. articulata* (1.71-fold induction) and two of its saponins (**1** and **3**) towards peroxisome proliferator-activated receptor-α may be beneficial in the cardiovascular condition that is closely associated with diabetes and other metabolic disorders.

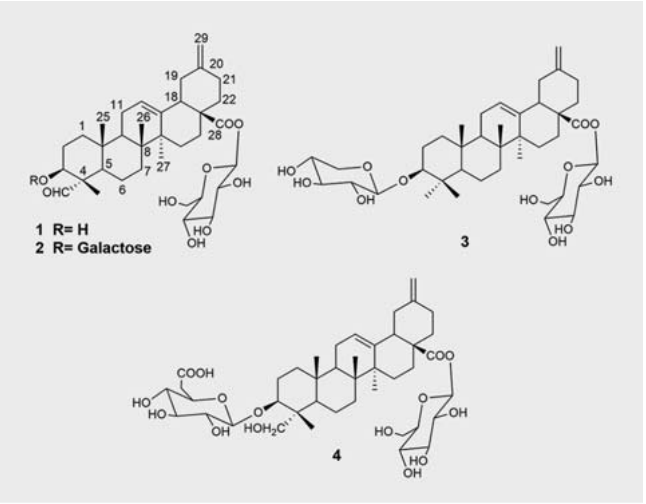
Introduction

Metabolic syndrome (MS) is characterized by increased abdominal fat, obesity, and insulin resistance. It occurs mainly due to the imbalance between high energy nutrition and physical activity [1]. The syndrome includes many metabolic disorders that predispose diabetes and cardiovascular complications [2]. Therefore, MS patients are at a high risk of cardiovascular disease (CVD), type II diabetes, diabetic nephropathy, and retinopathy. In this context, the peroxisome proliferator-activated receptor (PPAR) family has received increasing attention. PPARs constitute a subfamily within the nuclear receptor superfamily of ligand-inducible transcription factors [3]. There are three known subtypes of PPARs: α, β/δ, and γ [4]. They control the expression of genes involved in lipid metabolism, inflammation, and adipogenesis. PPARα is expressed in the heart, liver, muscles, and kidneys. It is responsible for controlling lipid and lipoprotein metabolism [5, 6]. Fenofibrate and gemfibrozil are selective agonists for PPARα that are efficient in improving

dyslipidemia but are not considered antidiabetic agents [7]. PPAR β/δ is expressed throughout the body and controls lipid metabolism in adipose tissue. PPARγ is present in two isoforms, 1 and 2. PPARγ-1 is expressed in the large intestine, adipose tissue, liver, kidneys, and muscles, while PPARγ-2 is restricted only to brown adipose tissue [6, 8, 9]. Therefore, diseases such as type II diabetes, obesity, metabolic syndrome, inflammation. and cardiovascular disease could be treated using the compounds that modulate PPAR activity [10]. Drugs used in MS target different symptoms such as body weight, insulin resistance, hyperglycemia, dyslipidemia, CVD, or hypertension. Increasingly, herbal remedies are being adopted as a form of treatment due to their benefits, which include lower cost, lower risks of side effects, and being more effective at targeting multiple pathways. It is therefore not surprising that medicinal plant-based products have been reported as beneficial for the treatment of type II diabetes and CVD, which leads to the development of MS [11]. Many publications confirmed the activity of some medicinal plants as PPARα and PPARγ activators [12, 13]. Practical insights may therefore be gleaned through further scientific analysis of these herbal medicines, which may lead to the development of alternative drug therapies that can better manage diabetic complications [14]. *Anabasis articulata* (Forssk.) Moq. (Amaranthaceae), locally named "Ajrem", is used traditionally to treat diabetes across Algeria [15]. Decoction of its aerial parts was reported to manage hyperglycemia through increasing blood insulin and α-fetoprotein [16]. Moreover, the plant showed a significant anti-inflammatory effect through the reduction of prostaglandin (PGE_2) and TNF-α levels as well as inhibition of cyclooxygenase-2 (COX-2) activity [17]. A phytochemical investigation of the plant resulted in the isolation of triterpenoid saponins that were responsible for the antidiabetic activity of the plant [18]. They were identified as 3-*β*-*O*-D-glucopyranosyl (of stigmasterol, *β*-sitosterol, and sitostanol), 3-*β*-*O*-D-glucopyranosyl olean-12-ene-28-oic acid, and 3-*β*-*O*-D-glucopyranosyl olean-12-ene-28-oic acid-28-*O*-*β*-D-xylopyranosyl ester in addition to proceric acid [16]. In the course of our interest in MS and medicinal plants that could be used to prevent MS-related complications [19–21], the present study was carried out to evaluate the potential antidiabetic and cardiovascular effects of the *n*-butanol fraction as well as the isolated 30-nortriterpenoid saponins from the flowering aerial parts of *A. articulata* in terms of their abilities to activate PPARα and PPARγ in human hepatoma (Hep G2) cells as they are involved in the metabolism of lipids and carbohydrates, respectively. This will help to explore the usefulness of the plant saponins to ameliorate diabetic and cardiovascular complications.

Results and Discussion

A chemical investigation of the saponin content of *A. articulata* resulted in the isolation of three new (**1**–**3**) along with one known (**4**) saponin (▶ **Fig. 1**). Their structures were elucidated through ^1H and ^{13}C NMR, 2D spectroscopic, and HR-ESIMS analyses. Based on the reported data, compound **4** was identified as 3-*O*-*β*-D-glucuronopyranosyl-30-norhederagenin 12–20(29)-dien- 28-oic acid-28-*O*-*β*-D-glucopyranosyl ester (boussingoside E) [22].



▶ **Fig. 1** Structures of the isolated compounds (**1**–**4**).

Compound **1** was obtained as an optically active ($[\alpha]_D^{10}$ + 25 (*c* 0.1, MeOH)) white amorphous powder with a molecular formula of $C_{35}H_{52}O_9$, which was deduced from the HR-ESI-MS *m/z* 617.3652 $[M + H]^+$ in the positive ion mode and showed positive results in Molisch's and Liebermann-Burchard tests, suggesting that **1** was a triterpenoid saponin.

The ^1H NMR spectrum (▶ **Table 1**) confirmed the presence of a nor-oleanane system that was supported by the display of four tertiary angular methyl signals at δ_H 0.68 (3H, s, H_3-26), δ_H 0.87 (3H, s, H_3-24), δ_H 0.88 (3H, s, H_3-25), and δ_H 1.14 (3H, s, H_3-27) in addition to an aldehyde proton at δ_H 9.22 (1H, s, H_3-23) [23]. Moreover, the spectra showed two singlets at δ_H 4.61 (1H, s, H_a – 29) and 4.62 (1H, s, H_b – 29) corresponding to an exomethylene group and one *tri*-substituted olefinic proton at δ_H 5.24 (1H, m, H-12). The ^{13}C NMR spectrum displayed 35 carbon signals, of which 6 carbon signals were assigned to a monosaccharide moiety and 29 carbon signals were assigned to the triterpenoidal aglycone. The ^{13}C spectrum displayed two carbonyl signals at δ_C173.5 and 206.5, which were assigned to a carboxylic group (C-28) and an aldehyde group. Moreover, the spectra showed four methyl resonances at δ_C 7.8, 14.1, 15.6, and 24.6, which were assigned to C-24, C-25, C-26, and C-27, respectively, eleven methylenes, including an oxymethylene signal at δ_C 106.1, five methines (including one olefinic signal at δ_C120.9 and one oxymethine), and nine quaternary carbons (including a carboxy carbon forming an ester linkage, δ_C173.5 for C-28, two olefinic signals at δ_C141.9 and 147.0, and an aldehyde signal at δ_C 206.5). Taken together, these data were indicative of a typical Δ^{12} 30-noroleanolic acid-type triterpene aglycone bearing an aldehyde group that was previously identified [23].

The aldehyde group was confirmed to be at C-23 from the HMBC correlations. The aldehyde proton at δ_H 9.22 was correlated in the HMBC with a carbon at δ_C 54.1 (C-4) in addition to a correlation between H_3-24 at δ_H 0.87 and the carbon at δ_C 206.5 (C-23) (▶ **Fig. 2**). Moreover, the proton of the aldehydic group showed a strong correlation in the NOESY experiment with δ_H1.28 (1H, m,

▶ **Table 1** NMR spectral data of compounds **1–3** (600 and 150 MHz, DMSO-d_6, δ ppm).

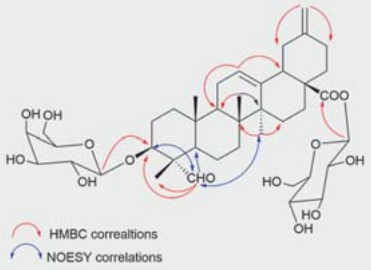
	1		**2**		**3**	
Position	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**
1	0.99, m, 1.56, m	36.6, CH_2	0.99, m, 1.56, m	36.6, CH_2	0.87, m, 1.48, m	37.1, CH_2
2	1.58, m, 1.90, m	24.9, CH_2	1.59, m, 1.96, m	23.1, CH_2	1.48, m, 1.85, m	24.3, CH_2
3	3.66, (brd, 11.5)	69.4, CH	3.78, (brd, 11.1)	79.2, CH	2.99, (brd, 11.9)	86.8, CH
4	–	54.1, C	–	53.4, C	–	37.7, C
5	1.28, m	45.2, CH	1.25, m	45.3, CH	0.69, m	53.9, CH
6	0.74, m, 1.37, m	19.2, CH_2	0.77, m, 1.34, m	18.5, CH_2	1.62, m, 1.42, m	16.7, CH_2
7	1.08, m, 1.40, m	30.3, CH_2	1.10, m, 1.40, m	30.4, CH_2	1.2, m, 1.38, m	31.2, CH_2
8	–	39.1, C	–	39.1, C	–	37.9, C
9	1.52, brs	45.9, CH	1.59, brs	45.8, CH	1.47, m	46.0, CH
10	–	34.3, C	–	34.3, C	–	35.2, C
11	1.82, m, 2.22, m	21.9, CH_2	1.83, m, 2.3, m	21.9, CH_2	1.84, m, 1.84, m	21.9, CH_2
12	5.24, m	120.9, CH	5.24, m	120.9, CH	5.21, m	121.1, CH
13	–	141.9, C	–	141.8, C	–	141.7, C
14	–	40.3, C	–	40.3, C	–	40.2, C
15	0.93, m, 1.74, m	26.1, CH_2	0.97, m, 1.73, m	26.1, CH_2	0.99, m, 1.74, m	26.1, CH_2
16	1.66, m, 2.10, m	21.6, CH_2	1.69, m, 2.08, m	21.5, CH_2	1.69, m, 2.07, m	21.6, CH_2
17		45.2, C		45.2, C		45.2, C
18	2.62, (dd, 13.6, 5.1)	45.6, CH	2.62, (brd, 13.6)	45.6, CH	2.61, (dd, 13.6, 6.5)	45.6, CH
19	2.01, (dd, 13.3, 6.5), 2.42, m	39.7, CH_2	2.01, (brd, 13.3), 2.45, m	39.8, CH_2	2.02, (dd, 13.6, 5.0), 2.45, m	39.9, CH_2
20	–	147.0, C		146.7, C		147.0, C
21	2.05, m, 2.14, m	28.2, CH_2	2.07, m, 2.18, m	28.2, CH_2	2.06, m, 2.15, m	28.2, CH_2
22	1.33, m, 1.76, m	35.7, CH_2	1.37, m, 1.78, m	35.6, CH_2	1.38, m, 1.7, m	35.6, CH_2
23	9.22, s	206.5, C	9.33, s	205.1, C	0.95, s	26.6, CH_3
24	0.87, s	7.8, CH_3	0.97, s	8.75, CH_3	0.74, s	15.4, CH_3
25	0.88, s	14.1, CH_3	0.89, s	14.2, CH_3	0.86, s	14.2, CH_3
26	0.68, s	15.6, CH_3	0.68, s	15.6, CH_3	0.68, s	15.6, CH_3
27	1.14, s	24.6, CH_3	1.13, s	24.5, CH_3	1.10, s	24.4, CH_3
28	–	173.5, C	–	173.5, C	–	173.5, C
29	4.61, s 4.62, s	106.1, CH_2	4.60, s 4.62, s	106, CH_2	4.57, s 4.55, s	106.0, CH_2
30			–	–	–	–
	***28-O-β*-D- glucopyranosyl**		***3β-O*-D-galactopyranosyl**		***3β-O*-D-xylopyranosyl-**	
1′	5.19, (d, *J* = 7.6)	93.1, CH	3.96, (d, *J* = 7.2)	101.8, CH	4.12, (d, 7.2)	104.3, CH
2′	3.09, (dd, 9.2, 7.8)	71.3, CH	2.79, m	72.6, CH	2.92, dd, (9.1, 7.6)	72.8, CH
3′	3.18, (t, 9.2)	75.6, CH	3.01, m	75.8, CH	3.09, m	75.7, CH
4′	3.11, (t, 8.7)	68.4, CH	2.99, m	71.0, CH	3.41, m	71.2, CH
5′	3.13, m	76.7, CH	3.07, m	72.6, CH	3.2, (dd, 11.4, 10.4), 3.3, (dd, 11.4, 5.4)	62.0, CH_2
6′	3.60, (dd, 11.8, 1.5), 3.43, (dd, 11.8, 4.8)	59.6, CH_2	3.26, (dd, 11.7, 4.7), 3.34, (dd, 11.8, 1.6)	62.4, CH_2	–	–
			***28-O-β*-D-glucopyranosyl**		***28-O-β*-D-glucopyranosyl**	
1″			5.24, (d, *J* = 7.6)	93.1, CH	5.19, (d, *J* = 7.6)	93.1, CH
2″			3.07, m	72.2, CH	3.07, (dd, 9.1, 7.6)	71.3, CH
3″			3.18, m	75.5, CH	3.19, m	75.6, CH
4″			3.08, (t, 8.7)	68.5, CH	3.08, (t, 8.8)	68.5, CH
5″			3.13, (t, 9.2)	76.7, CH	3.12, m	76.7, CH
6″			3.58, (dd, 11.8, 1.5), 3.41, (dd, 11.8, 5.1)	59.6, CH_2	3.58, (dd, 11.9, 1.7), 3.41, (dd, 11.9, 4.9)	59.5, CH_2

H-5) and H-3 at δ_H 3.66, which confirm the alpha orientation of the aldehydic group at C-23. The hydroxyl group at C-3 was confirmed to be β oriented through the NOESY correlation of H-3 at δ_H 3.66 and H-5 at δ_H 1.28, which confirmed that H-3 was assigned to be in an α-axial position.

Moreover, the ^{1}H spectrum showed an anomeric proton signal at δ_H 5.19 (▶ **Table 1**). The sugar moiety was identified as the β-D-glucopyranosyl moiety based on its $J_{H\text{-}1,\ H\text{-}2}$ coupling constant at δ_H 7.6 Hz [23]. Furthermore, the monosaccharide structure bonding to the 28-position of **1** was characterized by the HMBC experiment. Namely, a long-range correlation was observed between the anomeric proton H-1′ at δ_H 5.19 (1H, d, J = 7.6 Hz) of the glucopyranosyl moiety and C-28 (the carboxyl carbon at δ_C 173.5) of the noroleanane aglycone part. The acid hydrolysis of **1** revealed that it contains a D-glucose moiety. These results were confirmed through HPLC analysis of the hydrolytic products against different monosaccharaides, where the sugar has the same retention time as authentic D-glucose (30.7 min). In addition, all sugar protons and carbons were confirmed by extensive HSQC and HMBC analyses. Therefore, the structure of **1** was deduced as 3-β-hydroxy-23-aldehyde-30-norolean-12,20(29)-dien-28-oic acid-28-*O*-β-D-glucopyranosyl ester.

Compound **2** was obtained as an optically active ($[\alpha]_D^{10}$ + 15 (*c* 0.1, MeOH)) white amorphous powder with a molecular formula of $C_{41}H_{62}O_{14}$, which was deduced from the HR-ESI-MS *m/z* 779.4639 $[M + H]^+$ in the positive ion mode and showed a positive test for a triterpenoidal saponin. Its spectroscopic data were very similar to those of **1**. Detailed comparison of their 1D NMR data indicated that the differences between these compounds are in the positions and the number of sugar moieties. The molecular formula of **2** was identified as $C_{41}H_{62}O_{14}$ on the basis of the HR-ESI-MS, and gave D-glucose and D-galactose on acid hydrolysis. The aglycone part showed the same spectra as that of **1**, and it could be identified as 23-aldehyde-30-norolean-12,20(29)-diene. The ^{1}H and ^{13}C NMR spectra showed similar signals to those in **1** with two doublet signals integrating as one proton each, at δ_H 5.24 (d, J = 7.6 Hz) and 3.96 (d, J = 7.2 Hz) corresponding to two sugar moieties at C 28 and C3, respectively, as well as twelve carbon signals (▶ **Table 1**), which is in good agreement with the published data for the two sugar moieties [24]. The sugar moieties were identified as *O*-β-D-galactopyranosyl and *O*-β-D-glucopyranosyl moieties after careful inspection of the ^{1}H, ^{13}C NMR, and HMBC data and acid hydrolysis of **2**. Furthermore, the positions of the functional sugar moieties were confirmed by the HMBC experiment (▶ **Fig. 2**) to be attached to C-3 and C-28, respectively, from correlations between H-1′ (δ_H 3.96, d, J = 7.2 Hz) with C-3 at δ_C 79.2 and H-1″ (δ_H 5.24, d, J = 7.6 Hz) with C-28 at δ_C 173.5. Based upon all of the above evidences, the structure of **2** was elucidated as 3-*O*-β-D-galactopyranosyl-30-norolean-12,20(29)-dien-23-aldehyde-28-oic acid-28-*O*-β-D-glucopyranosyl ester.

Compound **3** was obtained as an optically active ($[\alpha]_D^{10}$ + 30 (*c* 0.1, MeOH)) white amorphous powder with a molecular formula of $C_{40}H_{62}O_{12}$ as inferred from the HR-ESI-MS *m/z* 735.4358 $[M + H]^+$ in the positive ion mode and showing the same pattern as compounds **1** and **2**. Its molecular formula was determined on the basis of the HR-ESI-MS pseudo-molecular ion peak at *m/z* 735.4358 $[M + H]^+$ (calcd. for 735.4366, $C_{40}H_{63}O_{12}$). The ^{1}H



▶ **Fig. 2** Some important HMBC and NOESY correlations for **1** and **2**.

NMR spectrum exhibited the following signals: five tertiary methyl signals at δ_H 0.68, 0.74, 0.86, 0.95, and 1.10, an olefinic proton signal at δ_H 5.21 (m, H-12), proton signals of an exomethylene group at δ_H 4.57 and 4.55 (each a singlet), and a signal typical of H-3*ax* (brd, δ_H 2.99) consistent with the presence of a β-OR group at C-3 position. In addition, the spectra displayed two anomeric protons at δ_H 5.19 (d, J = 7.6 Hz) and 4.12 (d, J = 7.2 Hz). The aglycone part was previously identified [25] and showed the same spectra as that of **1** and **2** except for the absence of the aldehyde proton signal and the presence of the normal pattern of the H_3-23 and H_3-24 methyl groups attached to C-4, and it could be identified as 30-norolean-12,20 (29)-diene.

The ^{13}C NMR spectrum (▶ **Table 1**) of **3** displayed 40 carbon signals. The aglycone moiety was assigned by 29 carbons, i.e., 8 quaternary carbons, 5 methines, 11 methylenes, and 5 methyls. In addition, the aglycone part showed four olefinic carbon signals (δ_C 121.1, 141.7, 147.0, and 106.0), one carbonyl carbon signal (δ_C 173.5), and an oxymethine carbon signal (δ_C 86.8). Thus, the spectral data, after comparison with previously published data [26], confirmed the presence of a triterpene glycoside with a 30-norolean-12–20(29)-dien-28-oic acid framework.

The glycosidic part was represented by 11 carbon resonances (one pentose and one hexose) as obtained by subtracting the 29 aglycone carbons from the total (40 signals). In addition, the two sugar moieties were represented by two anomeric carbons at δ_C104.3 and 93.1. The sugars were identified as β-D-glucopyranosyl and β-D-xylopyranosyl after careful inspection of ^{1}H, ^{13}C NMR, extensive 2D NMR analysis, and acid hydrolysis of **3** [27]. The location of the β-D-glucopyranosyl moiety at C-28 was confirmed by a significant deshielding of the anomeric proton H-1″ [δ_H 5.19 (d, J = 7.6 Hz); H-1″*ax*], and the signal at δ_C 93.1 is consistent with the presence of a sugar moiety attached to aglycone by an ester linkage [28]. This was confirmed by long-range coupling between hydrogen H-1″ (δ 5.19) and carbon C-28 (δ_C 173.5). All the previous data confirmed that **3** was 3-*O*-β-D-xylopyranosyl-30-norolean-12–20(29)-dien-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**).

The *in vitro* antidiabetic and cardiovascular activities of the *n*-butanol fraction as well as the isolated saponins were tested in terms of PPARα and PPARγ activation through a reporter gene assay with ciprofibrate and rosiglitazone as positive controls for PPARα and PPARγ, respectively. The *n*-butanol fraction was found

▶ **Table 2** Effect of the *n*-butanol fraction (total saponins) and isolated compounds from *A. articulata* on PPARα and PPARγ transcriptional activity as determined by the luciferase assay in HepG2 cells. Fold induction was calculated as the ratio of luciferase expression in sample-treated cells to the vehicle-treated cells. Data are presented as the mean ± SD of values from two independent experiments performed with two replicates each time.

Compound	Fold induction in PPARα activity			Fold induction in PPARγ activity		
	50 µM	25 µM	12.5 µM	50 µM	25 µM	12.5 µM
1	2.25 ± 0.12	1.74 ± 0.10	1.55 ± 0.19	1.48 ± 0.35	1.30 ± 0.16	1.17 ± 0.01
2	1.19 ± 0.14	1.60 ± 0.08	1.30 ± 0.20	1.4 ± 0.30	1.65 ± 0.15	1.60 ± 0.13
3	1.86 ± 0.02	2.08 ± 0.23	1.54 ± 0.26	1.53 ± 0.14	1.32 ± 0.03	1.28 ± 0.07
4	1.20 ± 0.22	1.30 ± 0.20	1.09 ± 0.08	1.50 ± 0.25	1.32 ± 0.02	1.3 ± 0.19
Ciprofibrate[a]	4.12 ± 0.39	2.97 ± 0.93	1.67 ± 0.12	–	–	–
Rosiglitazone[a]	–	–	–	4.36 ± 0.79	4.31 ± 0.11	4.71 ± 0.05
***n*-butanol fraction**[b]	1.71 ± 0.21	1.57 ± 0.28	1.67 ± 0.12	1.27 ± 0.17	1.19 ± 0.13	1.06 ± 0.01

[a] The test concentrations for the positive controls were 10, 5, and 2.5 µM. [b] The test concentrations for the *n*-butanol fractions (total saponins) were 100, 50, and 25 µg/mL. Fold induction = luciferase expression in sample-treated cells/luciferase expression in vehicle-treated cells

to exhibit a stronger agonistic effect towards PPARα with a fold induction of 1.71 (indicating a 71% increase in its activity) compared to the vehicle treated control. On the other hand, the activation of PPARγ was only 1.27-fold by the *n*-butanol fraction, representing an increase of only 27% (▶ **Table 2**).

Among the isolates, compounds **1** and **3** exhibited a stronger agonistic effect towards PPARα, significantly enhancing PPARα-directed luciferase expression with an increase of 2.25- and 1.86-fold, respectively, compared to the vehicle control. Compound **2** showed a lower increase in the activity of PPARα, while compound **4** did not show any effect (▶ **Table 2**). Compounds **1**, **3**, and **4** did not show > 1.5-fold increase in PPARγ activity, while **2** showed 1.6-fold activation of PPARγ, as shown in ▶ **Table 2**.

This class of compounds, nor-oleanane-type triterpenes, were reported for cytotoxic [23], hemolytic [29], and α-glucosidase inhibition [30] activities earlier. The reported hypoglycemic effect of the plant could be due to α-glucosidase inhibitory activity of its saponins. Salicornia saponin from *Salicornia bigelovii* Torr. (Amaranthaceae), which is very similar to **2**, is formulated for treating obesity and hyperlipidemia in the clinic, and processed to obtain a slimming health care food [31]. Moreover, boussingoside E (**4**), a triterpenoid saponin isolated from the tubers of *Anredera baselloides* (Kunth) Baill. (Basellaceae), was also reported to exhibit hypoglycemic activity [32].

It was reported previously that the triterpenoidal compounds and their glycosides exhibited a strong antidiabetic effect, especially those of the oleanane nucleus [33]. In addition, it was reported that oleanolic acid may improve cardiac lipid metabolism in Zucker Diabetic Fatty (ZDF) rats by acting on PPARα. Two glycosylated oleanolic acid derivatives from *Acer pictum* Thunb. (Sapindaceae) achieved a transactivation of the three PPAR subtypes, and they are very interesting for the treatment of metabolic diseases because they could simultaneously target insulin resistance, atherogenic dyslipemia, and obesity [34].

As a comprehensive interpretation, the structure-activity relationship of the isolated saponins on PPARα was found to be dependent on the pattern of C-23 and the mono and bidesmoside character of the isolated saponins as well as the type of sugars in the bidesmoside pattern. Compound **1** as a monodesmoside saponin esterified at C-28 with a glucose unit, in addition to the presence of methyl C-24 and an aldehydic group at C-23 instead of a methyl group, exhibited the most potent effect on PPARα. Alteration of this pattern by retaining the dimethyl pattern of C-23 and C-24 and adding a sugar at C-3 exhibited a lower effect, as in **3**, followed by the bidesmoside pattern (i.e., compound **2**). Moreover, a hydroxyl methyl pattern at C-23 in bidesmoside saponins exhibited the loss of activity on PPARα (compound **4**). These observations were consistent with the results obtained previously by Wang et al. [35], who showed that the saponins containing a methyl moiety at C-23 exhibit better activity than those with a hydroxyl methyl pattern at C-23. Therefore, the pattern of the ring A in the 30-noroleanene nuclei could affect the activity on PPARα, whether the compound shows a monodesmoside or a bidesmoside pattern. Replacement of the methyl at C-23 with an aldehyde or its substitution with a hydroxyl methyl pattern may result in modification of the activity.

In conclusion, a chemical investigation of *A. articulata*, which has previously shown antidiabetic activity through increasing the insulin level, resulted in the isolation of four major saponins. Due to the PPARα agonistic activity of these compounds, they can also serve as therapeutic agents for treating hyperlipidemic conditions associated with diabetes and obesity. Further investigation of the hypolipidemic action of compounds **1** and **3** is warranted.

Materials and Methods

General experimental procedures

An LTQ Orbitrap mass spectrometer (Thermo Finnigan) was operated to record HR-ESI-MS. An AUTOPOL IV Automatic Polarimeter (Rudolph) was used to determine specific rotations. 1D and 2D NMR spectra were recorded on Bruker DRX-850 and 600 MHz Ultrashield spectrometers (BrukerBioSpin) using DMSO as the solvent. TLC analysis was performed on precoated TLC plates with silica gel 60 F_{254} (Merck). Separation of pure compounds was performed on column chromatography using silica gel 60 (70–230

mesh, Merck), RP_{18} (0.04–0.063 mm Merck), and Sephadex LH-20 (Merck). Finally, purification of the compounds was done using a 6-mL standard LiChrolut extraction tube (RP_{18}, 40–63 µm; Merck).

The drug controls, ciprofibrate and rosiglitazone (purity > 98 %), were obtained from Cayman Chemical. DMEM, bovine calf serum (BCS), FBS, and PBS were from Hyclone. Penicillin/streptomycin and trypsin were purchased from Gibco. Two plasmids, pSG5-PPAR*α* (plasmid 22 751) and PPRE X3-tkluc (plasmid 1015), were from Addgene, while two others, pCMV-rPPARγ and pPPREaP2-tk-luc, were obtained from Dr. Dennis Feller, University of Mississippi (Department of Pharmacology). The test compounds were dissolved in DMSO at a 10 mM concentration, while the *n*-butanol fraction was dissolved at 20 mg/mL in DMSO.

Plant material

In December 2014, flowering aerial parts of *A. articulata* (Forssk.) Moq. were brought from Saudi Arabia. Dr. Emad Al-Sharif, (Faculty of Science & Arts, Khulais, King Abdulaziz University, Saudi Arabia) kindly identified the plant material. A specimen (AA1011) of the plant was deposited at the Herbarium of the Faculty of Pharmacy, King Abdulaziz University.

Extraction and isolation

Dried flowering aerial parts of *A. articulata* (1 kg) were extracted with methanol (3 × 3 L) at room temperature using an Ultraturrex homogenizer until exhaustion, followed by evaporation of the solvent under vacuum to give a semisolid dark brown residue (120 g, 12 %). The resulting residue was suspended in distilled water (500 mL) and partitioned successively with $CHCl_3$ (500 mL × 5), EtOAc (500 mL × 5), and *n*-butanol (150 mL × 5) to yield 50, 10, and 15 g, respectively. The *n*-butanol fraction (15 g) was chromatographed over silica gel 60 H for CC (5 × 100 cm) and eluted with $CHCl_3$ containing increasing amounts of MeOH and water to obtain seven fractions (fractions 1 to 7).

Fraction 3 (0.98 g) was chromatographed on a Sephadex LH-20 column (100 g, MeOH; 300 mL), resulting in four subfractions (fraction 3–1 → fraction 3–4). Subfraction 3–3 (220 mg) was further chromatographed on a reversed-phase silica gel column (50 g, 50 µm) using H_2O : MeOH (4 : 6, v/v; 120 mL) as an eluting system to yield compound **1** (10 mg). Fraction 4 (1.2 g) was chromatographed over a Sephadex LH-20 as fraction 3, affording five subfractions (subfraction 4–1 → subfraction 4–5). Subfraction 4–4 (350 mg) was chromatographed on a reversed-phase silica gel column using H_2O : MeOH (6 : 4, v/v; 150 mL) as an eluting system, followed by purification on preparative HPLC using an H_2O : MeOH (7 : 3, v/v) mixture to yield compounds **2** (15 mg) and **3** (12 mg). Finally, fraction 5 (500 mg) was chromatographed on RP-C_{18} (50 g, 50 µm) using H_2O : MeOH (1 : 1, v/v; 150 mL) to give four major subfractions (subfraction 5–1 → subfraction 5–4). Subfraction 5–2 (100 mg) was further purified on RP-C_{18} using H_2O : MeOH (7 : 3, v/v; 100 mL) to afford compound **4** (22 mg).

3β-Hydroxy,23-aldehyde-30-norolean-12,20(29)-dien-28-oic acid-28-O-β-*D-glucopyranosyl ester* (**1**): white amorphous powder; $[\alpha]_D^{10}$ + 25(*c* 0.1, MeOH); ^1H (DMSO, 600 MHz) and ^{13}C NMR (DMSO, 150 MHz); for data see ▶ **Table 1**; HRESIMS *m/z* 617.3652 $[M + H]^+$ (calcd. for 617.3658, $C_{35}H_{53}O_9$).

3β-O-D-Galactopyranosyl-23-aldehyde-30-norolean-12,20(29)-dien-28-oic acid-28-O-β-*D-glucopyranosyl ester* (**2**): white amorphous powder; $[\alpha]_D^{10}$ + 15 (*c* 0.1, MeOH); ^1H (DMSO, 600 MHz) and ^{13}C NMR (DMSO, 150 MHz); for data see ▶ **Table 1**; HRESIMS *m/z* 779.4639 $[M + H]^+$ (calcd. for 779.4672, $C_{41}H_{63}O_{14}$).

3β-O-D-Xylopyranosyl-30-norolean-12,20(29)-dien-28-oic acid 28-O-β-*D-glucopyranosyl ester* (**3**): white amorphous powder; $[\alpha]_D^{10}$ + 30 (*c* 0.1, MeOH); ^1H (DMSO, 600 MHz) and ^{13}C NMR (DMSO, 150 MHz); for data see ▶ **Table 1**; HRESIMS *m/z* 735.4358 $[M + H]^+$ (calcd. for 735.4366, $C_{40}H_{63}O_{12}$).

Determination of the absolute configuration of sugars

Hydrolysis of the isolated compound (1 mg) was performed by 2 M HCl in water (0.5 mL) and refluxing at 95 °C for 2 h. Then, NH_4OH was added for neutralization of the reaction mixture and the aglycone was extracted three times with EtOAc, leaving the sugars in the aqueous phase. The aqueous layer was lyophilized, and the dried residue was dissolved in pyridine (0.5 mL) and mixed with 1 mL of L-cysteine methyl ester hydrochloride in pyridine (0.1 M). Next, the mixture was heated at 90 °C for 1 h. Then, phenyl isothiocyanate in pyridine (1 mL) was added and the mixture was heated for 1 h at 90 °C. A reversed-phased HPLC [Waters Alliance 2795, equipped with a photodiode array detector and Luna C18 column (150 × 4.6 mm, 5 µm particle size; Phenomenex, Inc.)] was used to analyze the resulting compound. Two solvent systems were used for elution, the first being water containing 0.1 % acetic acid (A) and the second being acetonitrile containing 0.1 % acetic acid (B). They were injected in a gradient mode: A/B was mixed 90/10 for the first 20 min and then mixed at 45/55 for the next 25 min at a rate of 1 mL/min. The response was detected at 254 nm. The standard sugar derivatives were identically prepared and analyzed. The sugars were identified as D-glucose, D-galactose, and D-xylose by comparison of the retention time of their derivative with that of the authentic sugars [D-glucose: 30.7 min, D-galactose: 34.2 min, D-xylose: 33.9 min].

Determination of transcriptional activity of peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ by the reporter gene assay

Human hepatoma (HepG2) cells (ATCC) were grown in DMEM supplemented with 10 % FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. The detailed procedure has been reported earlier [36]. In brief, HepG2 cells were transfected by electroporation using the Square electroporator T820 (BTX) and its associated BTX disposable cuvette at 160 V for a single 70 ms pulse. Either a combination of pSG5-PPAR*α* and PPRE X3-tk-luc or a combination of pCMV-rPPARγ and pPPREaP2-tk-luc plasmid DNA (25 µg of each/1.5 mL cell suspension) was transfected into the cells by electroporation. The transfected cells were grown for a period of 24 h, having been plated at a density of 5×10^4 cells/well in 96-well tissue culture plates. After this time, test compounds (12.5, 25, and 50 µM) or drug controls (ciprofibrate or rosiglitazone; 2.5, 5.0, and 10 µM) were added to treat the cells for 24 h. At the end of this incubation period, the cells were lysed and a luciferase assay system (Promega) was employed to measure the luciferase activity. The highest concentration of DMSO (vehicle

control) was 0.5%. The effect of the test compounds on PPARα and PPARγ was determined in terms of fold induction in their activity, which was calculated as the ratio of the luciferase expression in sample-treated cells to the luciferase expression in vehicle-treated cells.

Statistical analysis

The fold induction values represented in ▶ **Table 2** are the average ± SD of two independent experiments performed with two replicates each time. Calculations were done using Microsoft Excel version 14.6.5.

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1–3** and their HSQC, HMBC, and NOESY correlations are available as Supporting Information.

Conflict of Interest

The authors declare no conflict of interest

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